Hybrid Calcium Phosphate Neuron-Like Structures under the Microscope

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Many biominerals display unique morphologies where inorganic building blocks interplay with organic matter. Organic molecules dictate the complex and sophisticated shapes of biominerals which take place at ambient conditions [1]. The close interplay between organic and inorganic components endows biominerals with unique architectures and exceptional properties [2,3]. The calcium phosphate system is of special interest due to its widespread appearance in biomineralization, especially in the formation of teeth and bones. However several fundamental aspects are still not clearly understood. Amorphous calcium phosphate as a precursor for biomineralisation is still being under consideration. Detecting early stages of biomineralisation is a real challenge. Due to unstable nature of amorphous calcium phosphate phases no unambiguous proof of its presence was shown until now.

For our study, different organic molecules were used to produce stabilized very unique calcium phosphate neuron-like structures [4]. Electron energy-loss spectroscopy (EELS) and energy-dispersive X-ray spectroscopy (EDX) combined with scanning transmission electron microscopy (STEM) imaging at high spatial and high energy resolution, as well as energy-filtered TEM (EFTEM) were used to characterize these structures using Zeiss SESAM and JEOL ARM200F microscopes at different accelerating voltages.

Lower magnification annular dark-field (ADF)-STEM images of typical calcium phosphate neuron-like structures are shown in Figure 1. The dense core of the structure and the filaments are clearly visible. According to our high-resolution TEM and electron diffraction data the structures are amorphous. EFTEM experiments using the low-loss EELS region were conducted on several neuron-like structures. A bright-field (BF)-TEM image and a corresponding calcium (Ca) map are shown in Figure 2. The intensity of the background corrected Ca- $M_{2,3}$ edges in the energy range between 32 and 40 eV was used to form the Ca map. In the course of our work also low-loss and core-loss EELS experiments were performed on these neuron-like structures as well as on standard materials and will be discussed.

References:

[1] S Weiner and PM Dove in "An Overview of Biomineralisation Processes and the Problem of the Vital Effects" (2003), Rev Mineral Geochem 54, 1-29.

- [2] UGK Wegst and MF Ashby, Philos Mag **84** (2004), 2167.
- [3] AP Jackson and JFV Vincent, J Mater Sci 25 (1990), 3173.
- [4] M Espanol, ZT Zhao, J Almunia and M-P Ginebra, J Mater Chem B 2 (2014), 2020.



Figure 1. (a, b) ADF-STEM images of the calcium phosphate neuron-like structures.



Figure 2. (a) BF-TEM image of neuron-like structures with (b) corresponding Ca map obtained by using the background subtracted intensity of Ca- $M_{2,3}$ edges.